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Review

Structures, physico-chemical properties, production and (potential) applications of sucrose-derived α-D-glucans synthesized by glucansucrases

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ABSTRACT

Glycoside hydrolase family 70 (GH70) glucansucrases produce α-D-glucan polysaccharides (e.g. dextran), which have different linkage composition, branching degree and size distribution, and hold potential applications in food, cosmetic and medicine industry. In addition, GH70 branching sucrases add single α-(1→2) or α-(1→3) branches onto dextran, resulting in highly branched polysaccharides with "comb-like" structure. The physico-chemical properties of these α-D-glucans are highly influenced by their linkage compositions, branching degrees and sizes. Among these α-D-glucans, dextran is commercially applied as plasma expander and separation matrix based on extensive studies of its structure and physico-chemical properties. However, such detailed information is lacking for the other type of α-D-glucans. Aiming to stimulate the application of α-D-glucans produced by glucansucrases, we present an overview of the structures, production, physico-chemical properties and (potential) applications of these sucrose-derived α-D-glucan polysaccharides. We also discuss bottlenecks and future perspectives for the application of these α-D-glucan polysaccharides.

1. Introduction

In recent years, the ability of lactic acid bacteria to produce large amounts of α-D-glucan exopolysaccharides from sucrose has drawn strong attention for industrial applications (Badel, Bernardi, & Michaud, 2011; Gangoiti, Pijning, & Dijkhuizen, 2018; Korakli & Vogel, 2006; Monsan et al., 2001; Ryan, Ross, Fitzgerald, Caplice, & Stanton, 2015; Zannini, Waters, Coffey, & Arendt, 2015). These sucrose-derived α-D-glucan polysaccharides are highly valued products, which may find wide applications in food, medicine and cosmetics (Badel et al., 2011; Leemhuis et al., 2013; Monsan et al., 2001). Glycoside hydrolase family 70 (GH70) glucansucrase enzymes of lactic acid bacteria catalyze the synthesis of these α-D-glucans using the low cost sucrose as the only substrate (Leemhuis et al., 2013; Lombard, Golaconda Ramulu, Drula, Coutinho, & Henrissat, 2014; Meng et al., 2016; Monchois, Willemot, & Monsan, 1999). They differ from Leloir glycosyltransferase enzymes, which require expensive nucleotide-activated sugars (e.g. UDP-glucose) as donor substrates (Mestrom et al., 2019). Synthesis of α-D-glucans thus represents an avenue to use sucrose as raw material to produce value-added products.

Notably, the synthesis of α-D-glucans from sucrose in lactic acid bacteria generally only requires single GH70 glucansucrase enzyme, which employs the α-retaining double displacement mechanism (Korakli & Vogel, 2006; Leemhuis et al., 2013; Monchois, Remaud-Siméon, Russell, Monsan, & Willemot, 1997, 1999). The (α1↔β2) linkage of sucrose is firstly cleaved with the formation of a β-glucosyl-enyzme covalent intermediate and the release of fructose. In the subsequent step, the growing α-D-glucan chain attacks the β-glucosyl-enyzme covalent intermediate, resulting in the transfer of the glucosyl moiety to the non-reducing end of the α-D-glucan. Depending on the glucansucrase enzyme, a wide diversity of α-D-glucan polysaccharides can be produced, varying in linkage compositions, sizes and branching degrees (Korakli & Vogel, 2006; Leemhuis et al., 2013; Meng et al., 2016; Monchois et al., 1999). Based on the linkage composition (Fig. 1), these α-D-glucan polysaccharides are classified into dextran with mainly α-(1→6) linkages, mutan with predominate α-(1→3) linkages, alternan with alternating α-(1→6) and α-(1→3) linkages, reuteran with mainly α-(1→4) linkages. In addition, GH70 branching sucrases can add single α-(1→2) or α-(1→3) branched residue onto dextran acceptor, resulting in highly branched polysaccharides with comb-like structure (Moulis,

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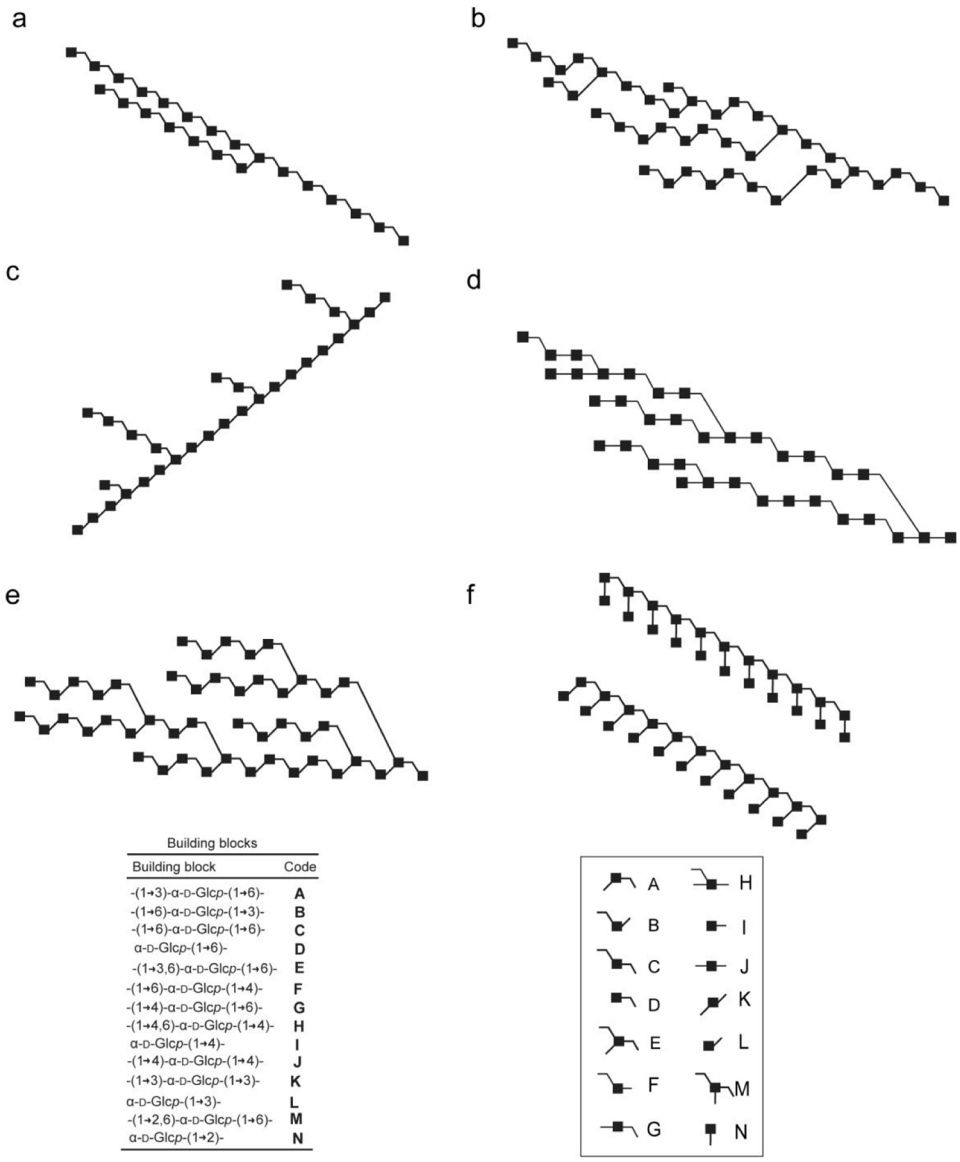

Fig. 1. Composite model for the dextran synthesized by *Leuconostoc mesenteroides* NRRL B-512F (a) (Naessens et al., 2005), dextran synthesized by *Lactobacillus reuteri* 180 (b) (van Leeuwen, Kralj et al., 2008b), mutan produced by *Streptococcus mutans* (c) (Davis et al., 1986; Naessens et al., 2005; Nisizawa et al., 1977), reuteran synthesized by *L. reuteri* 121 (d) (van Leeuwen, Kralj et al., 2008a), alternan produced by *L. mesenteroides* NRRL B-1355 (e) (Côté & Robyt, 1982; Naessens et al., 2005; Seymour et al., 1977), and highly α-(1→2) or α-(1→3) branched dextran with comb-like structure produced by branching sucrases (f) (Brison et al., 2013; Naessens et al., 2005; Vuillemin et al., 2016). Note: these composite models were built by incorporating all known structural units of these α-D-glucan polysaccharides to show their structural features and do not represent the actual structure of these α-D-glucan polysaccharides.

Andre, & Remaud-Simeon, 2016). For more details on the biosynthesis of α-D-glucans by glucansucrase enzymes, readers are directed to previously published excellent reviews (Gangoiti et al., 2018; Korakli & Vogel, 2006; Leemhuis et al., 2013; Meng et al., 2016; Moulis et al., 2016; Ryan et al., 2015; Zannini et al., 2015). Overall, GH70 glucansucrase enzymes are capable of producing α-D-glucans with different linkage composition and size using single enzymes incubated with sucrose, which is a great advantage for the production of tailor-made α-D-glucans (Leemhuis et al., 2013). However, the applications of these α-D-glucans are limited by the lack of data related to their detailed structure and physico-chemical properties. In order to stimulate the biotechnology community to further explore the production and application of these α-D-glucans, we present the structures, production, physico-chemical properties and (potential) applications of α-D-glucan polysaccharides synthesized by glucansucrase and branching sucrase enzymes from sucrose in this review (Table 1). In particular, we also discuss bottlenecks for the application of such polysaccharides and propose future researches that have to be addressed to promote the application of these α-D-glucan polysaccharides.

2. Brief overview of analytical methods for characterization of α-D-glucans produced by glucansucrases

The α-D-glucans synthesized *in vivo* by lactic acid bacteria fermentation or *in vitro* by glucansucrase enzymes at the lab scale are generally isolated, separated and purified by ethanol precipitation and size exclusion chromatography (van Geel-Schutten, Flesch, ten Brink, Smith, & Dijkhuizen, 1998). The purified α-D-glucans are firstly subjected to monosaccharide analysis, which can be achieved by complete hydrolysis of polysaccharides with sulfuric acid followed by high performance anion exchange chromatography (HPAEC) analysis (Miao, Bai et al., 2014). The monosaccharide analysis can also be performed by methanolysis followed by trimethylsilylation, generating a mixture of trimethylsilylated methyl glycosides, which can be analyzed by gas chromatography mass spectrometry (GC-MS) (van Leeuwen, Kralj et al., 2008b). After hydrolysis of polysaccharides, the monosaccharides can also be acetylated generating alditol acetate derivatives for GC–MS analysis (Pettolino, Walsh, Fincher, & Bacic, 2012).

The glycosidic linkage composition of purified α-D-glucans can be assessed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy analysis based on the structural-reporter-group concept established for α-D-glucans (van Leeuwen, Leeflang, Gerwig, & Kamerling, 2008). For a

Table 1
Typical α-D-glucans produced from sucrose by GH70 glucansucrases and branching sucrases, and their structural features, physico-chemical properties, applications and production.

Producing Microorganisms/ Enzymes	Type of α-D-glucans	Linkage composition	Reported physico-chemical properties	Applications	Production yields	References
L. mesenteroides NRRL B-512F/DSRS	Dextran	95% α-(1→6) and 5% α-(1→3)	Mw 4.01 × 10^8 Da, highly soluble in water	Plasma expander, Separation matrix, Bread baking, Potential prebiotics	135 g/L	(Irague et al., 2012; Karthikeyan et al., 1996; Naessens et al., 2005)
L. reuteri 180/Gtf180	Dextran	67% α-(1→6) and 33% α-(1→3)	Mw 2.26 × 10^7 Da,	NR	20 g/L	(van Geel-Schutten et al., 1998; van Leeuwen, Kralj et al., 2008b)
W. cibaria CMGDEX3	Dextran	96.6% α-(1→6) and 3.4% α-(1→3)	Mw > 2.0 × 10^6 Da	Sourdough baking	2.40 g/L	(Ahmed et al., 2012)
W. cibaria JAG8	Dextran	93% α-(1→6) and 7% α-(1→3)	Mw (NR), Solubility 24.5%, Water holding capacity 352%, Resistant to hydrolysis of gastric juice and α-amylase	Potential prebiotics (stimulating the growth of *B. animalis* subspecies *lactis*, *B. infantis* and *L. acidophilus*)	38 g/L	(Rao & Goyal, 2013; Tingirikari et al., 2014)
O. kitaharae DSM17330/DSR-OK	Dextran	98.2% α-(1→6) and 1.8% α-(1→3)	Mw > 10^9 Da, non-Newtonian properties, high viscosity, gel-like behavior	Potential texturing agent	NR	(Vuillemin et al., 2018)
S. mutans 6715	Mutan	33% α-(1→6) and 67% α-(1→3)	Water-insoluble	Pathogenic factor of dental caries	4.0 g/L	(Davis et al., 1986; Masumoto et al., 1987)
S. mutan OMZ176	Mutan	35% α-(1→6) and 65% α-(1→3)	Water-insoluble	Activation of the alternative pathway of the complement system in human serum	4.0 g/L	(Inal et al., 1976; Masumoto et al., 1987)
L. mesenteroides NRRL B-118/DsrI	Mutan	50% α-(1→6) and 50% α-(1→3)	Water insoluble, gel-forming	Potential application for bioplugging and encapsulation	NR	(Côté & Skory, 2012, 2015)
L. reuteri 35 − 5/GtfA	Reuteran	58% α-(1→4) and 42% α-(1→6)	Mw 3.47 × 10^7 Da,	Potential prebiotics	9.8 g/L	(Bai et al., 2016; van Leeuwen, Kralj et al., 2008a)
L. reuteri SK24.003	Reuteran	80% α-(1→4) and 20% α-(1→6)	Mw 4.31 × 10^7 Da, Solution with non-Newtonian pseudoplastic behavior	Potential prebiotics	40.8 g/L	(Miao, Ma, Huang et al., 2015; Miao, Ma, et al., 2014)
L. mesenteroides NRRL B-1355/ Alternansucrase	Alternan	60% α-(1→6) and 40% α-(1→3)	Low viscosity, high water solubility and strong resistance to enzymatic hydrolysis	Potential prebiotics	NR	(Côté & Robyt, 1982; Seymour et al., 1977)
L. citreum SK24.002	Alternan	57% α-(1→6) and 43% α-(1→3)	Mw 4.62 × 10^7 Da	Potential prebiotics (increased production of short chain fatty acids)	35 g/L	(Miao, Bai et al., 2014; Miao, Jia, Hamaker, et al., 2016)
L. citreum ABK-1/Alternansucrase LcALT	Alternan	60% α-(1→6) and 40% α-(1→3)	Forming 90 nm nanoparticles at low concentrations and opaque gel at high concentrations, Fragile biofilm formation	Drug encapsulation	NR	(Wangpaiboon et al., 2018)
L. citreum NRRL B-1299/DSRE-CD2 *L. citreum* NRRL B-1299 /Brs-A *L. kunkeei* EFB6/Brs-D	α-(1→2) branched dextran	0 − 50% α-(1→2) and 50 − 100% α-(1→6)	Resistant to hydrolysis by digestive enzymes	Potential prebiotics (stimulating the production of short chain fatty acids)	NR	(Brison et al., 2010; Passerini et al., 2015)
L. citreum NRRL B-742 /Brs-B *L. fallax* KCTC3537 /Brs-C *L. kunkeei* DSM 12361/GtfZ-CD2	α-(1→3) branched dextran	0 − 50% α-(1→3) and 50 − 100% α-(1→6)	Resistant to hydrolysis by digestive enzymes	Potential prebiotics (stimulating the production of short chain fatty acids)	NR	(Meng et al., 2018; Vuillemin et al., 2016)

NR: not reported.

more detailed analysis of the different linkages, α-D-glucans can be sent to methylation analysis to determine the ratio of different substituted monosaccharides (Ciucanu & Kerek, 1984; Pettolino et al., 2012), which is very useful for determining the relative amount of branching linkages present in the α-D-glucan polysaccharides. The determination of the exact structure of α-D-glucan polysaccharides can still not be achieved. In order to determine the structural units present in the α-D-glucan polysaccharides, they can be partially hydrolyzed by acid hydrolysis, resulting in a mixture of α-D-glucan oligosaccharides, which can be fractionated, purified, and further structurally analyzed by 1D and 2D ^{1}H-^{13}C NMR based on the structural-reporter-group, and matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Munkel et al., 2019; van Leeuwen, Kralj et al., 2008a, 2008b). α-D-Glucan polysaccharides can also be sent for 2D ^{1}H-^{13}C NMR analysis to determine the structural units present in the polysaccharides and their linkage pattern (van Leeuwen et al., 2009, 2008a, 2008b). Furthermore, α-D-glucan polysaccharides can also be hydrolyzed by specific enzymes, such as pullulanase, dextranase and α-amylases, generating a mixture of oligosaccharides, which can be analyzed by HPAEC (van Leeuwen, Kralj et al., 2008a). Based on all these structural information obtained from the above analysis, composite models can be built for α-D-glucan polysaccharides (Fig. 1), visualizing their structural features (van Leeuwen, Kralj et al., 2008a, 2008b). However, these composite models do not represent the actual structure of α-D-glucan polysaccharides. The size and dispersity of α-D-glucan polysaccharides can be determined either by a gel permeation/size exclusion chromatography (GPC-SEC) system with multiangle laser light scattering (MALLS) or asymmetrical flow field flow fractionation (AF4) with MALLS (Bai, Dobruchowska, van der Kaaij, Gerwig, & Dijkhuizen, 2016; Gaborieau & Castignolles, 2011; Irague et al., 2012; Miao, Ma, Huang et al., 2015; Striegel, Isenberg, & Côté, 2009). However, it has been reported that α-D-glucan polysaccharide (alternan) undergoes degradation during SEC analysis, resulting in inaccurate determination of the molecular weight of α-D-glucans (Striegel et al., 2009). The rheological properties of α-D-glucan polysaccharides can be determined by rheometer (Irague et al., 2012; Miao, Bai et al., 2014; Miao, Ma, Huang, et al., 2015). In addition, the nanoparticle size and surface morphology of α-D-glucan polysaccharides can be determined by dynamic light scattering (DLS) and scanning electron microscopy (Miao, Ma, Huang et al., 2015; Wangpaiboon et al., 2018).

3. Dextran

3.1. Structure of dextran

Dextran was first described by Pasteur in 1861, who found a microorganism-derived substance (dextran) being responsible for the gelification of sugarcane syrups (Monsan et al., 2001). Now, dextran is defined as a homopolysaccharide which is composed of D-glucose residues with mainly α-(1→6) linkages (more than 50%). Typically, dextran consists of consecutive α-(1→6) linkages in the backbone with branching linkage of mainly α-(1→3), and occasionally α-(1→2) and α-(1→4) linkages. The most widely studied dextran is the one produced by *Leuconostoc mesenteroides* NRRL B-512F, which consists of 95% α-(1→6) linkages and 5% α-(1→3) branching linkage (Fig. 1a, Table 1) (Monchois et al., 1997). There has been a controversy on the chain length of branches in dextran. Sequential degradation studies on the dextran produced by *L. mesenteroides* NRRL B-512F revealed that 40% of the branching side chains contain only one glucosyl unit; 45% of the branching side chains possess two glucosyl units, and the remaining are longer than two glucosyl units (Larm, Lindberg, & Svensson, 1971). Catalytic oxidation studies on the *L. mesenteroides* NRRL B-512F dextran, and dextran 10 and 80 produced by Pharmacia AB from graded hydrolysis of *L. mesenteroides* NRRL B-512F dextran showed that most of the branching side chains contain more than one glucose residue (Lindberg & Svensson, 1968). Enzymatic degradation (dextranglucosidase) of *L. mesenteroides* NRRL B-512F dextran demonstrated that this dextran may contain side chains up to 33 glucose residues (Walker & Pulkowni, 1973). Dextran may consist of a mixture of different molecules, containing either short or long branched side chains. Dextran produced by *Weissella* strains generally contain higher amounts of α-(1→6) linkages (> 97%) (Table 1) (Ahmed, Siddiqui, Arman, & Ahmed, 2012; Kang, Oh, & Kim, 2009; Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008). NMR analysis of the dextran produced by *Weissella confusa* C39-2 revealed the presence of 97.2% α-(1→6) linkages and 2.8% α-(1→3) linkages (Amari et al., 2013). Notably, dextransucrase of *Weissella cibaria* CMU (Kang et al., 2009) and glucansucrase DSRWC of *W. cibaria* (Kang et al., 2009), produce dextran with only repeating glucopyranosyl units linked by α-(1→6) linkages. Lactic acid bacteria isolated from sourdoughs, i.e. *Lactobacillus curvatus* 69B2 and *Leuconostoc lactis* 95A (Palomba et al., 2012), *Lactobacillus animalis* TMW 1.971 (Ruhmkorf et al., 2013), are also reported to produce dextrans with only α-(1→6) linkages. In contrast, glucansucrase Gtf180 of *Lactobacillus reuteri* 180 produced a dextran with a high proportion of α-(1→3) linkages (33%) and structural analysis revealed that the dextran produced by Gtf180 contained both [→3)Glc*p*(1→] and [→3,6)Glc*p*(1→] units, suggesting the presence of α-(1→3) linkages in the backbone and in branches (Fig. 1b), respectively (van Leeuwen, Kralj et al., 2008b). The sourdough isolate *Lactobacillus brevis* E25 was reported to produce a similar dextran as that of *L. reuteri* 180 (Dertli, Colquhoun, Cote, Le Gall, & Narbad, 2018). In addition, dextran branched with α-(1→4) linkages (17%) was reported to be produced by *Lactobacillus reuteri* TMW 1.106 (Ruhmkorf et al., 2013). *Leuconostoc citreum* B-2, isolated from a homemade fermentation product of pineapple, synthesized a highly branched dextran with 19% α-(1→3) linkages and a few α-(1→2) linkages (Feng et al., 2018).

Cryo-TEM and DLS analysis revealed that the dextran synthesized by dextransucrase of *L. mesenteroides* D9909 displayed well-defined spheroidal particles in solution, with diameters ranging from 100 to 450 nm (Semyonov, Ramon, Shoham, & Shimoni, 2014). The size of dextran particles are reported to be dependent on the enzyme and substrate sucrose concentration, and the reaction conditions (Semyonov et al., 2014). The truncated dextransucrase DSRS vardel △4 N of *L. mesenteroides* NRRL 512F synthesized dextran with a radius of gyration of 157 nm under its optimal reaction condition with 292 mM sucrose (Irague et al., 2012). In the same study, DSRS vardel △4 N has been engineered to generate several mutants, which produce dextran with a radius of gyration ranging from 55 to 206 nm and also with different ratios of α-(1→6)/α-(1→3) linkages. Three-dimensional structural modeling revealed that dextran showed a random coil conformation, containing less intra-molecular hydrogen bonds (Miao, Ma, Huang et al., 2015). The fine structure of dextrans, especially their conformations in solution, deserves further investigation.

3.2. Physico-chemical properties of dextran

The molecular weight of dextran produced by dextransucrase enzymes from different bacteria generally varies in the range of 9–500 MDa depending on the producing strains and enzymes. The size of dextran synthesized by DSRS of *L. mesenteroides* NRRL B-512F was determined to be over 10^8 Da (Irague et al., 2012). Seven different dextran polymers, with 3–20% α-(1→3) linkages, were produced by mutants of dextransucrase DSRS vardel △4 N of *L. mesenteroides* NRRL B-512F (Irague et al., 2013). The molar masses of these dextrans increase as the amount of α-(1→3) linkage decreases, ranging from 0.76 to 6.02 × 10^8 Da (Irague et al., 2012). The dextransucrase DSR-OK of *Oenococcus kitaharae* DSM17330 synthesizes a dextran of over 10^9 Da, which is the largest dextran reported to date (Vuillemin et al., 2018). Other typical glucansucrases are also efficient polymerases, producing dextran of high molar mass. Compared to DSRS, DSRM of *Leuconostoc citreum* NRRL B-1299 exclusively and efficiently catalyzes the synthesis of a linear dextran of low molar mass (27 kDa) (Claverie et al., 2017;

Passerini et al., 2015). The medical applications of dextran mainly require dextran polymer with low molar mass i.e. 10, 40 and 70 kDa, which are obtained by partial hydrolysis of native dextran and fractionation (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005; Spence, Higgins, & Kimmel, 1952). Direct control of the size of the dextran synthesized by glucansucrases enzymes would facilitate the production of dextran with desired size and properties. However, our understanding of the mechanism of the size control of glucansucrase polymer products is limited. Several studies have reported that domain V of glucansucrase proteins shows glucan-binding capacity and plays critical roles in the polymer size determination (Claverie et al., 2017, 2020; Meng et al., 2015; Moulis et al., 2006; Osorio, Zuniga, Mendoza, Jana, & Jimenez, 2019). Recently, amino acid residue close to catalytic site of enzyme was also found to be important determinants of the length distribution of the synthesized dextran (Claverie et al., 2019).

The α-(1→6) linked dextran polysaccharides represent very flexible and extended polymers that are generally highly soluble in water. The water solubility of different dextrans depends on their branched linkage pattern and degree of branching (Xu & Zhang, 2016). Linear dextrans, such as dextran produced by *L. mesenteroides* NRRL B-512F native enzyme, show higher solubility in water and their aqueous solutions display Newtonian fluid properties (Vuillemin et al., 2018). In contrast, some branched dextrans show non-Newtonian pseudoplastic behavior and shear rate thinning effects in rheological analysis, as revealed by a decrease of viscosity with an increase of shear rate (Das, Baruah, & Goyal, 2014). However, it was reported that a quasi linear dextran synthesized by DSR-OK of *O. kitaharae* DSM17330 [98.2 % α-(1→6) and 1.8 % α-(1→3) linkage] displayed non-Newtonian properties (Vuillemin et al., 2018). At low shear rates, its viscosity was found to be much higher than dextran produced by DSRS, probably due to its higher molecular weight (over 10^9 Da) and linearity (Vuillemin et al., 2018). High solubility (24.5%) and water holding capacity (352%, expressed as total weight after water adsorption *100%/total dry polysaccharide weight) were displayed by the dextran produced by dextransucrase from *Weissella cibaria* JAG8, suggesting its porous matrix structure (Tingirikari, Kothari, & Goyal, 2014). The molecular weight of the highly branched dextran produced by *Leuconostoc citreum* B-2 was 3.77 × 10^6 Da, and its water solubility index and water holding capacity were determined to be 80% and 450%, respectively, suggesting its texture improving properties (Feng et al., 2018). As a neutral polysaccharide, the viscosity of dextran is not influenced by pH or salt concentration but depends on the concentration and molecular weight of dextran, and temperatures (Xu & Zhang, 2016). The emulsion stability of *W. cibaria* JAG8 dextran was shown to be higher than that of guar gum and sodium alginate, implicating its potential use as emulsifier in food industry (Tingirikari et al., 2014). Dextran polymers with different percentages of α-(1→3) linkages (3%–20%) produced by mutants of dextransucrase DSRS vardel △4 N all demonstrate non-Newtonian and nonthixotropic behaviors in solution (Irague et al., 2012, 2013). Dextran Gcn1 with 3% α-(1→3) linkages displays a quasi-linear conformation while Gcn6 and Gcn7 with 15% and 20% α-(1→3) linkages, respectively, are the smallest and mostly densely branched (Irague et al., 2012). Interestingly, Gcn1 was found to be able to form rubber and flexible biofilms at ambient temperature without the addition of any plasticizer, supporting their use as novel biomaterials (Irague et al., 2012). The dextran produced by *L. mesenteroides* NRRL B-1498 was shown to display anti-corrosion properties, enabling its application in protecting steel in humid and acidic environments (Finkenstadt, Cote, & Willett, 2011). The physico-chemical properties of diversedextrans produced by a large panel of glucansucrases remain largely unknown, which has limited the broader application of dextrans.

3.3. Production of dextran

The majority of dextrans are synthesized by dextransucrases secreted by *Leuconostoc*, *Streptococcus*, *Weissella* and *Lactobacillus* species (Leemhuis et al., 2013; Meng et al., 2016; Monchois et al., 1999). The expression of dextransucrase by *Leuconostoc* is generally induced by sucrose while *Streptococcus* strains constitutively express dextransucrases (Neely & Nott, 1962). The commercial production of dextran is mainly achieved by culturing *L. mesenteroides* NRRL B-512F in batch with sucrose (Karthikeyan, Rakshit, & Baradarajan, 1996). Sucrose serves as carbon source for growth of *L. mesenteroides* NRRL B-512F, as inducer for dextransucrase expression and as substrate of dextransucrase for the synthesis of dextran. The produced dextrans are precipitated with ethanol or methanol from the viscous culture. After partial hydrolysis with diluted acid, dextrans of proper sizes are isolated by fractionation (Naessens et al., 2005). The effects of fermentation parameters, including pH, sucrose concentration, temperature, time and cation ions (calcium) on dextran yield have been investigated and optimized in detail (Hellman et al., 1955; Jeanes, Wilham, Tsuchiya, & Haynes, 1957; Neely, 1958; Robyt & Walseth, 1979; Tsuchiya et al., 1952, 1955). The yield of dextran reached 135 g/L using a strain of *L. mesenteroides* under optimized conditions (Karthikeyan et al., 1996). *L. citreum* B/110-1-2 has been employed for fermentative dextran production from sugarcane molasses in a plant, yielding 1 ton/day of technical grade dextran (Vidal et al., 2011). *W. cibaria* JAG8, isolated from apple, produced 38 g/L dextran in unoptimized media (Table 1) (Rao & Goyal, 2013). Cultivation of *L. curvatus* TMW 1.624, *L. reuteri* TMW 1.106 and *L. animalis* TMW 1.971 in Homohiochii medium containing 50 g/L sucrose produced 10.13 g/L, 9.24 g/L and 3.11 g/L α-D-glucan polysaccharides, respectively (Ruhmkorf et al., 2013). An isolate of *Pediococcus pentosaceus* was reported to produce linear dextran with a yield of 10.2 g/L (Patel, Kasoju, Bora, & Goyal, 2010). Cultivating *L. citreum* B-2 in Man-Rogosa-Sharpe (MRS) medium with 75 g/L sucrose gave a yield of 28.3 g/L highly branched dextran, reaching 76% theoretical yield (Feng et al., 2018).

It is common that lactic acid bacteria encode multiple glucansucrase enzymes and the production of dextran is prone to be affected by culturing conditions (Meng et al., 2016; Munkel et al., 2019; Passerini et al., 2015). Although commercial dextran has been produced by batch fermentation of *L. mesenteroides* NRRL B-512F, alternative cell-free enzymatic processes have been investigated (Monsan & Lopez, 1981; Monsan et al., 2001). Particularly, in the batch fermentation process, the pH has influence on the production of dextransucrase enzyme by *L. mesenteroides* NRRL B-512F and also on its activity and stability (Tsuchiya et al., 1952, 1955). Unfortunately, the optimum pH for enzyme production and for its activity and stability are not the same. Use of a cell-free enzymatic process could solve these problems. Furthermore, a cell-free enzymatic process allows tight control of different dextran biosynthesis parameters (temperature, pH, and enzyme to substrate ratio), resulting in products with lower dispersity. Hellman NN. et al. examined effects of the addition of certain oligosaccharides (i.e. maltose) or dextran of low molecular weight as primer, enzyme concentration, sucrose concentration, and temperature on the synthesis of clinical dextran (see below, Application of dextran) using a cell-free enzyme preparation (Hellman et al., 1955). In particular, they found that dextran of low molecular weight was preferable to maltose as primer and the molecular weight of dextran produced increased with the molecular weight of the primer. The sucrose concentration was also found to be critical for controlling the molecular sizes and degree of branching of dextran synthesized (Kim, Robyt, Lee, Lee, & Kim, 2003). At higher sucrose concentrations, the size of dextran produced decreased whereas the degree of branches increased. The cell-free enzymatic process also allows immobilization of dextransucrase enzymes (Kaboli & Reilly, 1980; Lopez & Monsan, 1980; Monsan & Lopez, 1981). Kaboli H. et al. investigated the properties of immobilized dextransucrase of *L. mesenteroides* NRRL B-512F and showed that there was no significant change compared to the free soluble enzyme (Kaboli & Reilly, 1980). Monsan P. et al. investigated the production of dextransucrase enzymes by *L. mesenteroides* NRRL B-512F in a semi-

continuous process with slow addition of concentrated sucrose and purified the dextransucrase enzymes from the fermentation broth (Monsan & Lopez, 1981). Then, dextransucrase enzymes were immobilized onto amino-Spherosil support activated with glutaraldehyde and clinical dextran with less dispersity was synthesized by the immobilized enzymes (Monsan & Lopez, 1981).

Except for partial acid hydrolysis, hydrolysis of dextran catalyzed by dextranase has also been explored for the production of dextran with specific size. Kim & Day assessed the production of clinical dextran by mixed-culture fermentation of *Lipomyces starkeyi* ATCC 74054 and *Leuconostoc mesenteroides* ATCC 10830, which produce dextranase and dextransucrase, respectively (Kim & Day, 1994). The dual enzyme system of dextransucrase and dextranase was also employed for the synthesis of dextran with different size (from 7 kDa to 20 kDa) (Gan, Zhang, Zhang, & Hu, 2014). However, the industry still employs the conventional fermentation of *L. mesenteroides* strains for the production of commercial dextran. Several reasons may account for not adopting enzyme-based processes for industrial dextran production. First, the production costs may not be reduced by use of the cell-free enzymatic system; in contrast, the extra step of dextransucrase synthesis even may increase the total cost. Second, the yield of dextran production may not increase. Third, a new process for the enzymatic synthesis and separation of dextran at industrial scale has to be developed, requiring strong efforts. Fourth, the safety data for products of such new cell-free enzymatic processes have to be collected and approved prior to their applications, potentially a lengthy and costly step.

3.4. Application of dextran

In the medical industry, dextrans with a molecular size between 40 − 100 kDa are used as blood plasma volume expander for emergency treatment of blood loss and are recognized as clinical dextran (Fig. 2) (Bergentz, 1978; Koster, Sele, Schwartz, & Sindrup, 1957; Naessens et al., 2005; Shoemaker, 1976; Spence et al., 1952). Clinical fractions of dextran with molecular weights of 70 000, 60 000 and 40 000 Da (designated dextran 70, 60 and 40) are at present available for replacing moderate blood losses (Naessens et al., 2005; Spence et al., 1952). *L. mesenteroides* NRRL B-512F has been selected for clinical dextran production, due to the better effects of this dextran as a plasma volume expander. The high percentage of α-(1→6) linkages in dextran produced by *L. mesenteroides* NRRL B-512F (95%) imparts it higher water solubility, lower viscosity and good water-holding capacity, which is desirable for blood plasma expander (Jeanes et al., 1954; Naessens et al., 2005). Dextran 70 and dextran 40 are used at 6% and 10% concentrations, respectively, in normal saline solution. To reduce the risk of anaphylactic reactions, a small volume of dextran with low molecular weight (1000 Da) is used prior to dextran 70 or dextran 40 (Richter & Hedin, 1982). For medical applications, dextran sulfate has also been investigated as a heparin substitute (anticoagulant), however, it only showed up to 15% of the activity of heparin (Ricketts, 1952; Ricketts et al., 1953).

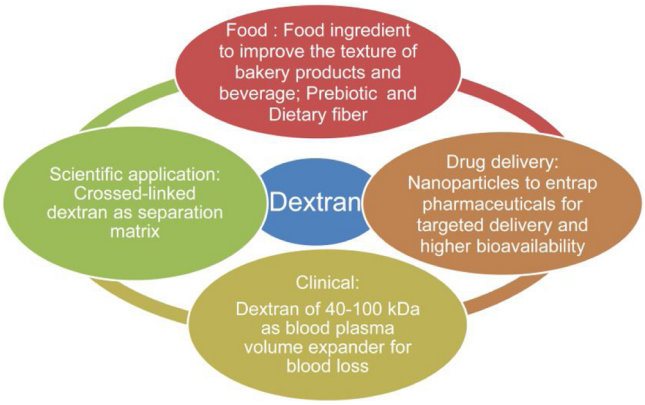

Fig. 2. Schematic representation of the most important applications of dextran produced by glucansucrase enzymes.

The use of dextran produced by *L. mesenteroides* NRRL B-512F as novel food ingredient in bakery has been authorized by the European Commission in 2000 (Naessens et al., 2005). Specifically, the use of dextran produced primarily by *L. mesenteroides* NRRL B-512F, which contains mainly linear α-(1→6) linkages and a few α-(1→3) branches with a size ranging from 1-2 MDa, in bread baking, has been authorized. Bakery products with dextran have improved softness and increased volume (Fig. 2) (Sandra et al., 2012). Specifically, dextran has been used to improve the texture and mouthfeel of gluten-free bread (Galle et al., 2012). The in situ production of dextran in sourdough has been used to improve the sourdough fermentation process and hence the quality of bread (Galle et al., 2012; Kajala et al., 2015; Katina et al., 2009; Sandra et al., 2012). Dextran was produced in wheat bran using dextransucrase Wc392-rDSR of *Weissella confusa* VTT E-90392, generating wheat bran containing 8.1% dry weight of dextran (Kajala et al., 2015). Supplementation of 20% such wheat bran with dextran in wheat baking resulted in significantly improved bread softness and reduced volume loss, clearly showing the potential of using dextran in baking (Kajala et al., 2015). The in situ produced dextran in traditional fermented beverage is important for the viscosity, texture, stability and mouthfeel of the products (Rao & Goyal, 2013; Waldherr, Doll, Meissner, & Vogel, 2010). Dextran was also shown to be a potential prebiotic or a dietary fiber in functional food (Olano-Martin, Mountzouris, Gibson, & Rastall, 2000; Tingirikari et al., 2014): the α-(1→6) linkages of dextran are resistant to digestive enzymes, however, dextran is fermentable by beneficial microbes. For example, the dextran produced by dextransucrase from *W. cibaria* JAG8 was resistant to hydrolysis by simulated gastric juice and digestion by α-amylase (Tingirikari et al., 2014). Growth of probiotic strains such as *Bifidobacterium animalis* subspecies *lactis*, *Bifidobacterium infantis* and *Lactobacillus acidophilus* was comparably stimulated by *W. cibaria* JAG8 dextran and commercial prebiotic inulin, while the growth of *Escherichia coli* was not enhanced (Tingirikari et al., 2014). The use of dextran in animal feed is also authorized to be generally regarded as safe (GRAS) status by the Food and Drug Administration (FDA). Many other potential food applications for dextran have been reported, e.g. as emulsifying and thickening agents, and as high-viscosity gums (Naessens et al., 2005).

Pharmacia cross-linked dextran using epichlorohydrin and glycerol, resulting in a product named Sephadex, which has been widely explored as separation matrix for the separation of proteins, carbohydrates and other biological molecules, based on their sizes, charges and other properties (Fig. 2) (Flodin, 1961; Naessens et al., 2005). It has also been shown that the conjugation of drugs, enzymes and other biological active molecules with dextran improves their span life time, stability, activity and targeted delivery (Herman, Persijn, Vandekerckhove, & Schacht, 1993; Molteni, 1985). Enzymatically synthesized dextran nanoparticles were also evaluated to entrap a hydrophobic nutraceutical, the isoflavone genistein (Semyonov et al., 2014). Under optimized conditions (pH 5.2–6 and sucrose concentration > 0.5 M), dextransucrase from *L. mesenteroides* D9909 synthesized spherical dextran nanoparticles (100–450 nm), which were shown to entrap 5.6 g genistein per 100 g particles, implying the potential use of dextran nanoparticles as a platform for delivering non-soluble bioactive chemicals in foods and/or pharmaceuticals (Fig. 2). Fang Y. et al. attached glucose or maltose as self-assembly monolayer on the gold surface and used the dextransucrase to elongate the attached glucose or maltose, forming the dextran brushes on the surface (Fang, Wu, & Xu, 2015). These polysaccharide brushes have great potential for biomedical applications, especially for detecting saccharide-protein interactions (Fang et al., 2015).

Various reports evaluate the global market for dextran, e.g. https://www.reportsanddata.com/report-detail/global-dextran-market-2017-

forecast-to-2022. The readers are directed to this report, which categorizes the dextran market based on manufacturers, regions, type and applications.

4. Other α-D-glucan polysaccharides synthesized by GH70 glucansucrases and branching sucrases from sucrose

4.1. Mutan

Mutans are water-insoluble glucopolysaccharides, consisting of mainly consecutive α-(1→3) linkages in the glucan chain backbone. However, methylation analysis showed that they also contain a minority of consecutive α-(1→6) linkages and α-(1→3,6)-D-Glc*p* branches (Nisizawa, Imai, & Araya, 1977). Mutans are generally produced by mutansucrase from *Streptococcus* strains and are associated with the development of dental caries (Bowen & Koo, 2011). Structural analysis of a water-insoluble α-D-glucan produced by *S. mutans* 6715 revealed the presence of continuous 67% α-(1→3) linkages in the backbone and 33% α-(1→6) linkages extending linearly from the branches (Fig. 1c, Table 1) (Davis, Hines, & Edwards, 1986). *Streptococcus salivarius* HHT produced a water-insoluble α-D-glucan containing high proportion of α-(1→3) linkages (80%) and short side chains of α-(1→4) and α-(1→6) linkages (Tsumuraya & Misaki, 1979). Various mutans (water-insoluble α-D-glucans) are also produced by *Leuconostoc* and *Lactobacillus* strains (Côté & Skory, 2012; Jeanes et al., 1954; Kralj et al., 2004; Pearce, Walker, Slodki, & Schuerch, 1990). DSRI of *L. mesenteroides* NRRL B-1118 produces a water-insoluble α-D-glucan, containing predominately α-(1→3) linkages (Table 1) (Côté & Skory, 2012). *L. reuteri* ML1 encodes a glucansucrase enzyme GtfML1, synthesizing a α-D-glucan with 65 % of α-(1→3) linkages (Kralj et al., 2004). Structural analysis of a α-D-glucan polysaccharide produced by a mutant strain of *L. mesenteroides* NRRL B-1355 (*L. mesenteroides* R1510) revealed the presence of linear α-(1→3) and α-(1→6) linkages, and branching α-(1→2) and α-(1→3) linkages (Côté, Ahlgren, & Smith, 1999). This α-D-glucan may be a graft polymer synthesized by combined actions of several glucansucrase enzymes, considering that a single glucansucrase generally catalyzes the synthesis of only two types of α-glycosidic linkage (Côté et al., 1999). The glucansucrases Lr1215 and Lr2135 of *L. citreum* TMW 2.1194 also catalyze the synthesis of water insoluble α-D-glucans with significant portions of consecutive α-(1→3) linkages (Munkel et al., 2019).

The physico-chemical properties of mutan have not been extensively studied due to its water insolubility. The hygroscopicity of the mutan powder produced by *S. mutans* was similar to that of corn starch, and the mutan solution displayed non-Newtonian and shear-thinning behavior (Masumoto et al., 1987). Scanning electron microscope examination of the water-insoluble α-D-glucan produced by *S. mutan* 20381 revealed a fibrillary structure consisting of granular formations (Wiater, Choma, & Szczodrak, 1999). Wiater A. et al. investigated the fermentative production of mutan using three different *S. mutan* strains (*S. mutan* 6067, *S. mutan* 20381 and *Streptococcus sobrinus* 6070) in four different media (complex and synthetic) (Wiater et al., 1999). *S. mutan* 20381 produced the largest amounts of water-insoluble α-D-glucans (about 400 mg/L) in brain-hear infusion broths (Wiater et al., 1999). The optimum conditions for the production of water-insoluble α-D-glucan (mutan) by *S. mutans* strains were determined as 15% (w/w) of sucrose, pH 7.5 and 37 °C, which yielded 4 g/L water insoluble α-D-glucan (Masumoto et al., 1987). Wiater A. et al. optimized the conditions of *Streptococcus sobrinus* CCUG 21020 mutan production, achieving a yield of 2.2 g/L (Wiater, Szczodrak, & Pleszczynska, 2005). *Lactobacillus satsumensis* NRRL B-59839, isolated from water kefir starter cultures, produced a significant amount of water-insoluble α-D-glucan (3.6 g/L) with high proportion of α-(1→3) linkages (about 40%) (Côté, Skory, Unser, & Rich, 2013). Using DrsI of *L. mesenteroides* NRRL B-1118, factors influencing the enzymatic synthesis of water-insolube α-D-glucan from sucrose were investigated (Côté & Skory, 2015). Both substrate and enzyme concentrations significantly affected the yield of water-insoluble α-D-glucan and the optimum sucrose concentration was determined to be 0.3 − 0.4 M with 1.7 U/mL of enzyme. Moreover, the addition of soluble dextran was also observed to increase the yield of insoluble α-D-glucan, probably because the added soluble dextran acted as the primer to stimulate the activity of DsrI and its α-D-glucan synthesis (Côté & Skory, 2015). Therefore, the above studies show that optimum conditions for mutan production are highly dependent on the particular enzymes and strains used. In order to produce insoluble mutans using cell-free enzymes, DsrI of *L. mesenteroides* was expressed as a secreted protein in *Lactococcus lactis* (Skory & Côté, 2015). The yield of the recombinant enzyme was 150-fold higher than the native *L. mesenteroides* strain, reaching 380 mg/L in the culture media. However, compared to dextran, the production of mutan has received less attention and to our best knowledge the water-insoluble mutan is not commercially available yet.

No specific commercial applications have been developed for mutans yet, but also have not been explored to any great extent. However, mutans are major dental caries pathogenic factors, and their roles in biofilm formation have been extensively studied (Bowen & Koo, 2011). One example is the mutan produced by *S. mutans*, one of the main components of dental caries biofilms (Emilson, Nilsson, & Bowen, 1984). *S. mutans* strains with a high mutansucrase activity were more cariogenic than those with little mutansucrase activity (Wenham, Davies, & Cole, 1981). The water-insoluble mutans facilitate adherence of microorganisms to smooth surfaces (e.g. tooth enamel). GtfB (formerly known as Gtf-I) and GtfC (Gtf-SI) of *S. mutans* synthesize water-insoluble α-D-glucans with mainly α-(1→3) linkages (Shiroza, Ueda, & Kuramitsu, 1987; Ueda, Shiroza, & Kuramitsu, 1988). *S. mutans* also produces a third glucansucrase enzyme [GtfD (Gtf-S)], catalyzing the synthesis of water-soluble α-D-glucan with mainly α-(1→6) linkages (Hanada & Kuramitsu, 1989). Inactivation of any of these three glucansucrase enzymes resulted in a reduction of smooth-surface carious lesions in the specific pathogen-free rat model system (Yamashita, Bowen, Burne, & Kuramitsu, 1993). Using Gtf-deficient *S. mutan* mutants, it was reported that the sucrose-dependent adherence to tooth surface was related to all 3 Gtf enzymes at an optimum ratio (Ooshima et al., 2001). Interestingly, most *Streptococcus* strains (i.e. *S. sobrinus* 6715 and *Streptococcus downei* Mfe28) encode both a glucansucrase producing water-insoluble α-D-glucans and a glucansucrase producing water-soluble α-D-glucans (Gilmore, Russell, & Ferretti, 1990; Walker, Cheetham, Taylor, Pearce, & Slodki, 1990). Apparently, the presence of both these water-(in)soluble α-D-glucans enhances the adherence of the microorganism to the smooth surface. Recently, Hyun-Jung Kwon et al. showed that mutan produced by *S. mutans* promoted alveolar bone loss in rat maxillae, which suggest a molecular mechanism for the inflammation induced by *S. mutans* during the establishment of periodontal disease (Kwon et al., 2016). Favorable biological activity of mutan has also been reported. It was shown that the water-insoluble α-D-glucan produced by *S. mutan* OMZ176 stimulated the alternative pathway of the complement system in human serum for immunomodulatory protein activation (Table 1) (Inal, Nagaki, Ebisu, Kato, & Kotani, 1976). Both the consecutive α-(1→3) backbone (65%) and the side chains containing α-(1→6) linkages (35%) were essential for the activation of the complement system. Considering the water insolubility of mutan, some other applications can be anticipated, including its use as a carrier for encapsulation technology, bioplugging and the production of biocompatible films and fibers (Côté & Skory, 2015).

4.2. Reuteran

Reuterans are glucopolysaccharides consisting of mainly consecutive α-(1→4) linkages and single α-(1→6) linkages in the glucan chain backbone, and α-(1→4,6) branches (Fig. 1d). Structural analysis of the reuteran produced by GtfA of *L. reuteri* 121 revealed the presence of 58% α-(1→4) and 42% α-(1→6) linkages, with a size of 34.6 MDa

(Table 1) (Bai et al., 2016; van Leeuwen, Kralj et al., 2008a). It is built up from maltose, maltotriose, and maltotetraose elements with single α-(1→6) linkages, showing mainly a glucose backbone with alternating α-(1→4) and α-(1→6) linkages, and 4,6-substituted branches (Fig. 1d) (van Leeuwen, Kralj et al., 2008a). The terminal residues are mostly α-D-Glc*p*-(1→4)- units with minor amounts of α-D-Glc*p*-(1→6)- units. Methylation studies showed that this reuteran consists of terminal, 4-substituted, 6-substituted and 4,6-disubstituted α-D-glucosyl residues in a molar ratio of 14, 46, 26 and 14% (van Leeuwen, Kralj et al., 2008a). The reuteran polysaccharide produced by GtfO of *Lactobacillus reuteri* ATCC 55730 composed of 80% α-(1→4) linkages and 20% α-(1→6) linkages, suggesting the presence of large amounts of consecutive α-(1→4) linkages instead of alternating α-(1→4) and α-(1→6) linkages (Kralj et al., 2004). Similarly, the exopolysaccharide produced by *L. reuteri* SK24.003 possesses predominate α-(1→4) linkages (80%) and fewer α-(1→6) linkages (20%) with a size of 43.1 MDa and a radius of gyration of 43.6 nm (Table 1) (Miao, Ma et al., 2014). Scanning electron microscope analysis showed that *L. reuteri* SK24.003 α-D-glucan displayed like a cementitious material with a porous network form. Structural modelling of *L. reuteri* SK24.003 α-D-glucan demonstrated that its α-(1→4)/α-(1→6) backbone and branches packed into helical groove, generating a helical conformation in solution (Miao, Ma, Huang et al., 2015). The viscosity of *L. reuteri* SK24.003 α-D-glucan increased linearly at the concentration range of 3.0–20%, however, its viscosity is significantly lower than that of dextran (Miao, Ma, Huang et al., 2015). But at concentrations above 10%, the viscosity of *L. reuteri* SK24.003 α-D-glucan is much higher than that of arabic gum at equivalent concentrations. The solution of *L. reuteri* SK24.003 α-D-glucan exhibited non-Newtonian pseudoplastic behavior and the viscosity of *L. reuteri* SK24.003 α-D-glucan solution dramatically decreased with increasing shear rate from 0.01 to 100 L/s. The production of reuteran has been reported for a limited number of *L. reuteri* strains. Fermentation of *L. reuteri* SK24.003 in MRS medium with 100 g/L sucrose produced 40.8 g/L reuteran (Miao, Ma, Huang et al., 2015; Miao, Ma, et al., 2014) while cultivation of *L. reuteri* 35−5 (mutant of *L. reuteri* 121) in the same medium produced 9.8 g/L reuteran (Van Geel-Schutten et al., 1999).

Reuteran applications have not been comprehensively explored. However, reuteran has been reported to be a potential prebiotic. The *in vitro* fermentation of extracellular α-D-glucan produced by *L. reuteri* SK24.003 by human fecal microbiota remarkably increased the short chain fatty acid production, including acetic acid, propionic acid and n-butyric acid (Miao, Ma, Jiang et al., 2015). Notably, the production of n-butyric acid at 48 h fermentation reached 1.29 mM, a value higher than that seen with commercial prebiotic oligosaccharides (Miao, Ma, Jiang et al., 2015). The commensal gut microbial species *Bacteroides thetaiotaomicron*, equipped with abundant CAZymes (Lombard et al., 2014), was shown to only partially utilize the reuteran produced by *L. reuteri* 121 (van Bueren, Saraf, Martens, & Dijkhuizen, 2015), indicating that the alternating α-(1→4)/α-(1→6).backbone with branching linkages made the reuteran difficult to be degraded, requiring specific enzymes, such as pullulanases. Feeding the weanling pig with reuteran-producing *L. reuteri* significantly reduced the colonization of enterotoxigenic *Escherichia coli*, implicating the use of reuteran to improve the health of livestock (Yang, Galle, Le, Zijlstra, & Ganzle, 2015). The reuteran of *L. reuteri* TMW 1.656 was reported to display anti-adhesive properties, which reduce enterotoxigenic effects of *E.coli* in piglets (Chen, Woodward, Zijlstra, & Ganzle, 2014), and it was also shown to improve the bread volume and softness (Chen, Levy, & Ganzle, 2016).

4.3. Alternan

The production of alternan has mainly been reported for strains of *L. mesenteroides* and *L. citreum*. Initially, a glucansucrase from *L. mesenteroides* NRRL B-1355 was found to synthesize a high molecular weight polymer containing alternating α-(1→6) and α-(1→3) linkages (Fig. 1e) (Côté & Robyt, 1982). Thus, this α-D-glucan was named alternan and its corresponding glucansucrase as alternansucrase (Côté & Robyt, 1982). Alternan is a branched α-D-glucan with 7–11 % 3,6-substituted-D-glucosyl residues (Seymour, Slodki, Plattner, & Jeanes, 1977). *L. citreum* ABK-1 also encodes an alternansucrase LcALT, which catalyzes the synthesis of a α-D-glucan with 60% α-(1→6) linkages and 40% α-(1→3) linkages (Table 1) (Wangpaiboon et al., 2018). Structural analysis by 2D NMR and methylation analysis showed that α-D-glucan synthesized by LcALT contains irregularly alternating α-(1→6)-linked and α-(1→3)-linked glucosyl residues. Alternan-producing *L. mesenteroides* and *L. citreum* strains have also been isolated from sourdoughs and the genes encoding alternansucrases were confirmed by partial sequence analysis (Bounaix et al., 2010). *L. citreum* SK24.002, isolated from Chinese traditional pickled vegetables, was found to produce a α-D-glucan polysaccharide with α-(1→6)-substituted and α-(1→3)-substituted glucopyranosyl residues at a ratio of 5:4 (Table 1) (Miao, Bai et al., 2014; Miao, Jia, Jiang, et al., 2016). Molecular characterization using 2D NMR spectroscopy confirmed the presence of predominately alternating α-(1→6) and α-(1→3) linkages (Miao, Jia, Hamaker et al., 2016). *L. citreum* L3C1E7, isolated from Pico cheese, produced alternan with a similar ratio of α-(1→6) and α-(1→3) linkages to that of *L. citreum* SK24.002 (Domingos-Lopes, Lamosa, Stanton, Ross, & Silva, 2018). The molecular size of *L. citreum* SK24.002 and *L. citreum* L3C1E7 alternans were determined as 46.2 MDa and 5.88 MDa, respectively (Table 1) (Domingos-Lopes et al., 2018; Miao, Jia, Jiang et al., 2016).

L. citreum SK24.002 was reported to produce 35 g/L alternan in MRS containing 100 g/L sucrose (Table 1) (Miao, Bai et al., 2014) while *L. citreum* L3C1E7 only produced 515.8 mg/L alternan in MRS with 50 g/L sucrose (Domingos-Lopes et al., 2018). Alternan has also been produced in potato tubers by expressing the *asr* gene of *L. mesenteroides* NRRL B-1355 in potato, yielding 1.2 mg/g fresh weight (Kok-Jacon et al., 2007).

Alternan displays low viscosity, high water solubility and strong resistance to enzymatic hydrolysis (Côté, 1992), making it suitable for the production of ingredients for functional foods such as potential prebiotics. *In vitro* fermentation of the *L. citreum* SK24.002 α-D-glucan polysaccharides by human faecal microbiota significantly increased the bacterial population and reduced the pH of fermentation by producing short chain fatty acids (Table 1) (Miao, Jia, Hamaker et al., 2016). The radius of gyration of *L. citreum* SK24.002 α-D-glucan was determined to be 37.8 nm (Miao, Bai et al., 2014). At low concentrations (< 10%) in aqueous solution, the alternan produced by LcALT of *L. citreum* ABK-1 undergoes a self-assembly process to form nanoparticles with an average size of 90 nm (Wangpaiboon et al., 2018). At increasing concentrations of the LcALT alternan, the viscosity of the solution increased. At concentrations above 12.5%, the nanoparticles formed by *L. citreum* ABK-1 alternan disassembled and an opaque gel was formed. The formation of nanoparticles or an opaque gel thus can be interconverted in an alternan concentration dependent manner, which makes alternan a very promising candidate for drug encapsulation applications (Wangpaiboon et al., 2018). In addition, a clear transparent film was formed upon drying the LcALT alternan solution, although it was fragile. Truncation of 3 and 7 of the SH3-like repeats in the C-terminus of the LcALT (△3SHALT and △7SHALT) protein had no influence on the linkage composition of alternan products (Wangpaiboon et al., 2019). However, the average nanoparticle size of alternan produced by both truncation mutants reduced to 80 nm (Wangpaiboon et al., 2019). The concentrations, required for forming the gel like states, increased to 15% and 20%, respectively, for the △3SHALT and △7SHALT polymer (Wangpaiboon et al., 2019). These results imply that subtle differences in the structure of the α-D-glucan produced can significantly alter their physico-chemical properties. Treatment of alternan with ultrasonification or spores from *Penicillium* spp. reduced its molecular weight and generated alternan derivatives with similar rheological properties to those of commercial arabic gum, providing new applications for alternan (Côté, 1992; Leathers, Naunnally, & Cote, 2002; Leathers, Nunnally, Ahlgren, & Cote, 2003; Leathers, Nunnally, &

Cote, 2009). In addition, alternan has been shown to promote the cell proliferation, migration and differentiation of human mesenchymal stem cells (Charoenwongpaiboon et al., 2019), showing promising medical applications of alternan.

4.4. Highly branched α-D-glucans synthesized by GH70 branching sucrases

In addition to typical glucansucrases, the GH70 family also hosts a distinct group of enzymes, designated branching sucrases (Moulis et al., 2016). Unlike typical glucansucrases, branching sucrases mainly catalyze the hydrolysis of sucrose and do not synthesize α-D-glucan polysaccharides when only sucrose is present (Moulis et al., 2016). Typically, upon the addition of dextran as acceptor substrate, branching sucrases catalyze the synthesis of α-(1→2) or α-(1→3) linked single glucosyl unit branches onto dextran, generating highly branched (up to 50%) dextran with "comb" like structure (Fig. 1f) (Moulis et al., 2016). Initially, *L. citreum* NRRL B-1299 was shown to synthesize dextran containing up to 35% α-(1→2) linked single-glucosyl-unit branches (Kobayashi & Matsuda, 1977). The glucansucrase DSRE encoded by *L. citreum* NRRL B-1299 was shown to contain two catalytic domains (CD1 and CD2) interconnected by a central glucan-binding domain (GBD) (Bozonnet et al., 2002). DSRE-CD1 was proven to be a dextransucrase while DSRE-CD2 was characterized as branching sucrase, catalyzing the synthesis of α-(1→2) branches (Table 1) (Fabre et al., 2005). Recently, five other branching sucrases with similar activity were identified by genome sequencing and database mining. Among these, Brs-B of *Leuconostoc citreum* NRRL B-742 (Vuillemin et al., 2016), Brs-C of *Leuconostoc fallax* KCTC3537 (Vuillemin et al., 2016) and GtfZ-CD2 of *Lactobacillus kunkeei* DSM 12361 (Meng et al., 2018) were found to synthesize α-(1→3) branches while Brs-A of *L. citreum* NRRL B-1299 (Passerini et al., 2015) and Brs-D of *Lactobacillus kunkeei* EFB6 (Passerini et al., 2015) catalyze the synthesis of α-(1→2) branches (Table 1). Importantly, the percentage of α-(1→2) or α-(1→3) branches in the products synthesized by these branching sucrases can be kinetically controlled by varying the [Sucrose]/[Dextran] ratio (Brison et al., 2010; Vuillemin et al., 2016). For example, using DsrE-CD2, dextrans with controlled amounts of α-(1→2) branches ranging from 13 to 40% were synthesized by varying the initial molar ratio of [Sucrose]/[Dextran] from 0.12 to 4.74 (Brison et al., 2010). Structural analysis of branched glucooligosaccharide products of DSRE-CD2 revealed that it catalyzes branching at random positions of dextran and eventually can glucosylate vicinal α-(1→6)-linked D-Glc*p* units through the formation of α-(1→2) linkages (Brison et al., 2013). The synthesis of α-(1→2) branched glucooligosaccharides from sucrose and maltose by *L. citreum* NRRL B-1299 was optimized and the [Sucrose/Maltose] ratio was determined to be essential parameters for the yield of branched glucooligosaccharides (Dols-Lafargue, Willemot, Monsan, & Remaud-Simeon, 2001).

The presence of α-(1→2) or α-(1→3) branches in branched dextrans renders them resistant to the hydrolysis by digestive enzymes of both humans and animals (Sarbini et al., 2011; Valette et al., 1993). Moreover, the resistance to hydrolysis by digestive enzymes i.e. glucoamylase and endodextranase, increased at higher α-(1→2) or α-(1→3) branching percentages (Brison et al., 2013). Instead, beneficial intestinal microbiota, e.g., *Bifidobacteria*, *Lactobacilli* and *Bacteroides* are capable of metabolizing these branched dextrans while detrimental strains poorly metabolize these α-D-glucans (Djouzi et al., 1995; Sarbini et al., 2011). In addition, the fermentation of these branched dextrans considerably increased the production of short chain fatty acids, such as acetate, propionate and butyrate (Sarbini et al., 2011). These characteristics illustrate that highly branched dextrans are potential prebiotics to improve intestinal health. The combination of dextransucrase DSR-OK from *O. kitaharae* DSM17330 with branching sucrase Brs-A from *L. citreum* NRRL B-1299 or Brs-B△1 from *L. citreum* NRRL B-742 produced new branched dextrans with high molecular weight (over 10^9 Da), which showed good film-forming properties (Faucard et al., 2018).

5. Prospectives

α-D-Glucan polysaccharides produced by GH70 glucansucrases derived from lactic acid bacteria hold great potential for applications in the food, cosmetic and medicine industry. A variety of α-D-glucans with different linkage compositions, branching degrees and sizes has been reported (Meng et al., 2016; Monchois et al., 1999; Monsan et al., 2001; Moulis et al., 2016). Among these α-D-glucans, only dextrans synthesized by DSRS from *L. mesenteroides* NRRL B-512F are commercialized, mainly for analytical and therapeutic applications (Naessens et al., 2005). This is probably because, on the one hand, dextran is the most studied α-D-glucan polysaccharide with respect to both structure and physico-chemical properties. On the other hand, the larger scale production of dextran by fermentation has been developed. This facilitates fundamental and applied research towards its properties, and its application at industrial scale. The applications of other type of α-D-glucans (mutan, reuteran, alternan and highly branched α-D-glucan) produced by glucansucrase enzymes are limited by the lack of data related to their detailed structure and physico-chemical properties. Expanding the knowledge of the physico-chemical properties of these α-D-glucans is urgently needed and may pave the way for their rational applications. Especially, the highly branched "comb-like" α-D-glucans synthesized by branching sucrases may show novel physico-chemical properties (Moulis et al., 2016). The relatively low yields currently achieved for production of other α-D-glucans is another important cause for lack of their commercial exploration. The production of various α-D-glucans in large quantities and reliable quality at sufficient low cost remains to be developed for successful applications. For the development of commercial production of α-D-glucans by enzymatic synthesis, efficient expression systems for glucansucrase enzymes are required. Moreover, the GRAS status of most α-D-glucan-producing lactic acid bacteria raises the potential of in situ production of texturing and bioactive α-D-glucans for food applications, in which the production and purification of α-D-glucans is not needed.

α-D-Glucan polysaccharides produced from sucrose by glucansucrase enzymes have not been widely selected for commercial use. Among others, the most important factor is the price of α-D-glucan polysaccharides from sucrose. Innovations for higher-value added products like pharmaceutical and functional food application are necessary to promote the commercial utilization of α-D-glucan polysaccharides from sucrose. Exploring the nanoparticles formed from α-D-glucans for drug delivery represent promising high-value applications. In addition, accumulated evidence has shown that α-D-glucans produced by glucansucrases have prebiotic potential and may find applications as novel ingredients in functional food to improve intestinal health.

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CRediT authorship contribution statement

Xiaodan Li: Writing - original draft, Funding acquisition. **Xiaofei Wang:** Writing - original draft. **Xiangfeng Meng:** Conceptualization, Writing - original draft, Funding acquisition. **Lubbert Dijkhuizen:** Conceptualization, Writing - review & editing, Supervision. **Weifeng Liu:** Writing - review & editing, Funding acquisition, Supervision.

Declaration of Competing Interest

None.

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